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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Use of Interferon and a Substance with an Antimalarial Activity for the Treatment of Malaria Infections

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Abstract

Use of interferon and a substance with an antimalarial activity for the treatment of malaria infections

Clinical (erythrocytic) malaria may be treated by administering effective amounts of an interferon, e.g. IFN-γ, and at least one antimalarially active substance which is preferably selected from 9-aminoacridines, 4-aminoquinolines, 8-aminoquinolines, biguanides, diaminopyrimidines, quinine salts, sulphonamides, sulfanilamides, antibiotics and/or sulphones e.g. chloroquine [(7-chloro-4-(4-diethylamino-1-methylbutylamino)-quinoline].

Claims

1. A method of treating a subject suffering from clinical (erythrocytic) malaria which comprises administering effective amounts of an interferon and at least one antimalarially active substance to the subject.

2. A method as claimed in claim 1 wherein the anti malarially active substance is selected from 9-aminoacridines, 4-aminoquinolines, 8-aminoquinolines, biguanides, diaminopyrimidines, quinine salts, sulphonamides, sulfanilamides, antibiotics and/or sulphones.

3. A method as claimed in claim 1 or claim 2 wherein the antimalarially active substance is selected from the 4-aminoquinolines.

4. A method as claimed in any one of the preceding claims wherein the antimalarially active substance is chloroquine [(7-chloro-4-(4-diethylamino-1-methylbutylamino)-quinoline].

5. A method as claimed in any one of the preceding claims wherein the interferon is species-specific.

6. A method as claimed in claim 5 is human interferon.

7. A method as claimed in any one of the preceding claims wherein the interferon is IFN-γ.

8. Use of interferon and at least one substance with an antimalarial activity for preparing a pharmaceutical composition for treatment of erythrocytic clinical malaria.

9. Use as claimed in claim 8, wherein the agent with an antimalarial activity is a synthetic, semisynthetic or naturally occurring active substance.

10. Use as claimed in claim 8 or claim 9, wherein the antimalarially active substance is selected from 9-aminoacridines, 4-aminoquinolines, 8-aminoquinolines, biguanides, diaminopyrimidines, quinine salts, sulphonamides, sulfanilamides, antibiotics and/or sulphones.

11. Use as claimed in any one of claims 8 to 10, wherein the antimalarially active substance is selected from the 4-aminoquinolines.

12. Use as claimed in claim 11, wherein the antimalarially active substance is chloroquine [(7-chloro-4-(4-diethylamino-1-methylbutylamino)-quinoline].

13. Use as claimed in any one of claims 8 to 12, wherein the interferon is prepared from natural cells or by DNA-recombination.

14. Use as claimed in any one of claims 8 to 13, wherein the interferon is IFN-γ.

15. Use as claimed in any one of claims 8 to 14, wherein the interferon is species-specific.

16. Use as claimed in claim 15, wherein the interferon is a human interferon.

17. Use as claimed in claims 15, wherein the interferon is an animal interferon.

18. A pharmaceutical composition for the treatment of clinical (erythrocytic) malaria comprising interferon

and at least one antimalarially active substance.

19. A pharmaceutical composition as claimed in claim 18 wherein the malarially active substance is selected from 9-aminoacridines, 4-aminoquinolines, 8-aminoquinolines, biguanides, diaminopyrimidines, quinine salts, sulphonamides, sulfanilamides, antibiotics and/or sulphones.

20. A pharmaceutical composition as claimed in claim 18 or 19 wherein the malarially active substance is selected from the 4-aminoquinolines.

21. A pharmaceutical composition as claimed in claim 20 wherein the antimalarially active substance is chloroquine [(7-chloro-4-(4-diethylamino-1-methylbutylamino)-quinoline].

22. A pharmaceutical composition as claimed in any one of claims 18 to 21 wherein the interferon is IFN-γ.

23. A pharmaceutical composition as claimed in any one of claims 18 to 222 wherein said composition is provided as a two-part pack comprising (i) interferon and (ii) at least one malarially active substance.

24. A method as claimed in claim 1 substantially as hereinbefore described.

25. Use as claimed in claim 8 substantially as hereinbefore described.

26. A pharmaceutical composition as claimed in claim 18 substantially as hereinbefore described.

27. Each and every novel method, use, process and composition described herein.

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Use of interferon and a substance with an antimalarial activity for the treatment of malaria infections

The present invention relates to the use of interferon and an active substance with an antimalarial activity for treating malaria infections in the erythrocytic (clinical) phase. The invention also relates to the use of a pharmaceutical composition containing on at least one antimalarially active substance which additionally contains an interferon, preferably interferon-gamma (IFN-γ).

Malaria infections are caused by protozoa of the genus Plasmodium, e.g. those of the species Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale, which are known to infect human beings preferentially, as well as Plasmodium berghei, Plasmodium knowlesi, Plasmodium vinckei, Plasmodium cynomologi, Plasmodium chabaudi, Plasmodium yoellii, which attack chiefly animal hosts, e.g. rodents and monkeys.

Although malaria has been treated for decades partly by eliminating any intermediate host with insecticides and partly by therapeutic measures, this infective illness caused by various types of Plasmodium still constitutes one of the major health problems worldwide. It is estimated that, every year, up to 200 million people living in tropical countries are infected with malaria pathogens, with lethal consequences for 1 to 2 million of those affected (STURCHLER, D., Experientia 40, 1357-1362, 1984).

The problem of fighting malaria is chiefly that, when insecticides are used, insecticide-resistant intermediate hosts or vectors (Anopheles) occur again and again and the drug treatment, which is basically only a prophylactic, constantly allows drug-resistant

protozoa (Plasmodium) to develop (DOBERSTYN, E.B., Experientia 40, 1311-1317, 1984; BRUCE-CHWATT, L.J., Annu. Rev. Public Health 8, 75-110, 1987).

Ever since the start of modern malaria research initiated over 100 years ago with the discovery of the original pathogen causing malaria (LAVERAN, A., Bull, Acad. Méd. Paris 9, 1235-1236, 1980) it has not been possible to provide an active ingredient which provides an adequate and lasting therapy for malaria infection.

The bite of the infected mosquito causes malaria parasites (sporozoites) to be transferred to humans. These sporozoites colonise the liver where they multiply. At the end of this development phase, parasites (merozoites) enter the bloodstream and attack erythrocytes. The actual clinical phase of the illness begins with the known symptoms and complications on infection of erythrocytes.

Treatment of malaria in humans and animals has hitherto been of a purely prophylactic nature although a number of measures have been proposed which comprise the use of antimalarial substances, immunisation or vaccination and the use of cytokines.

The known antimalarial substances can be divided into the following 6 main groups on the basis of their chemical compositions:

1. the 9-aminoacridines (e.g. mepacrine),
2. the 4-aminoquinolines (e.g. amodiaquine, chloroquine, hydroxychloroquine),
3. the 8-aminoquinolines (e.g. primaquine, quinocide),
4. the biguanides with an inhibiting effect on dihydrofolic acid reductase (e.g. chloroproguanil, cycloguanil, proguanil),
5. the diaminopyrimidines (e.g. pyrimethamine),
6. the quinine salts.

In addition to these groups, sulphones such as

dapsone, sulphonamides, sulphanilamides and antibiotics such as tetracycline are also used as antimalarial agents.

Depending on their mode of activity the known antimalarial agents can be divided into the following categories:

1. causal prophylactic substances effective against primary tissue stages,
2. active substances directed against relapses or recurrences and effective against latent tissue stages,
3. blood schizonticides,
4. gametocytocides and
5. sporonticides.

The first group includes, for example, proguanil, pyrimethamine and primaquine and the derivatives thereof and possibly also sulphanilamides, sulphonamides and tetracyclines. For the second group, 8-aminoquinolines such as primaquine and its analogues and derivatives are available, for example, as well as floxacrine, cycloguanil, dapsone and quinazolines. Substances active against the blood schizonts, the third category, include in particular 4-aminoacridines such as mepacrine and the 4-aminoquinolines such as chloroquine or chloroquinesulphate, quinine, amodiaquine and mepacrine, mefloquine and related compounds such as halofantrene, as well as pyrimethamine, proguanil, primaquine and the sulphanilamides and sulphonamides, particularly in conjunction with pyrimethamine.

Other substances which may be considered are the sesquiterpene lactones based on the compound artemisinine and the semisynthetic derivatives thereof such as artesunate and artemether as well as piperaquine, hydroxypiperaquine, pyronaridine, halofantrene and, generally, the biguanides and quinine salts.

The schizonticides mentioned above are effective against the gametocytes of, for example, P. vivax, P. malariae or P. ovale, but not against the mature gametocytes. The 8-aminoquinolines such as primaquine and quinocide are also effective against the gametocytes. Proguanil, primaquine and pyrimethamine may be mentioned as sporonticidal agents. Other known antimalarial agents are: chloroproguanil, cycloguanil (e.g. as a salt of embonic acid), pamaquine, plasmocide, totaquine, spirogermanium, febrifugine, brusatol, bruceine-A, bruceine-B, bruceine-C, yadanziolide-A, tebuquine, enpirolin, eurycomanone, 3-(4-imidazolyl)-2-(pivaloylamido)propionylhydrazide, cinchonidine; cucurbitacine, tripynadine, 5-ethylthioribose, arteether (ethylether analogues of artemether), artenilic acid, pyrexol, atalaphillinine, diformyldapsone, bruceantine, nitroquine, octanoylprimaquine, pyrimethamine plus sulfadoxine, hivernine, dabequine, artelinic acid, mefloquinquinate, halfantrin-beta-glycerophosphate, nimbolide, sergeolide (quassinoid of Picrolemma pseudocoeffea), simalikalactone-D, fluoroquine, fluorenmethanol, isouramil, cycloleucine, acedapsone (diacetyldapsone), gentiopicrine, amquinate (amquinolate), endochine, pentaquine, isopentaquine, methylchloroquine, amopyroquine, quinine, hydroquinine (dihydroquinine), dimeplasmine, azacrine, diapromine, menoctone, cycloquine (haloquine), lapinone, aristoquine, cloguanamil, clociguanil, brindoxime, cinchonine, tripiperaquine, 3-hydroxy-2-(4-(4-phenyl)-cyclohexyl)-1,4-anthraquinone, aminodiaquine, 4-methyl-5-n-pentoxyprimaquine, 4-methyl-5-n-hexoxyprimaquine, 2-(4-(4-chlorophenyl)-cyclohexyl)-3-hydroxy-1,4-naphthalenedione, gossypol derivatives, halofantrine (1,3-dichloro-α-(2-(dibutylamino)ethyl)-6-(trifluoromethyl)-9-phentantrene-methanol), cinchona alkaloids (e.g. in the combination quinine, quinidine, cinchonine), N,N'-bis(3-((phenylmethyl)amino)propyl)-

1,8-octanediamine, N,N-bis(3-((phenyl-methyl)amino)-propyl)-1,7-diaminoheptane, selenium-analogues of 2-acetyl and 2-propionyl-pyridinethiosemicarbazones, tebuquine, 2,6-bis(1-piperidinylmethyl)-4-((7-(trifluoromethyl)-4-quinolinyl)amino)-phenol, primary phosphoric acid esters of 4'-chloro-5-(1,1-dimethylethyl)-3-(((1,1-dimethylethyl)amino)methyl)-(1,1'-biphenyl-2-ol, N4-(2,6-dimethoxy-4-methyl-5-(3-trifluoromethyl)-phenoxy-8-quinolinyl)-1,4-pentanediamine, N,N-diethyl-N'-(6-methoxy-4-methyl-8-quinolinyl)-1,6-hexanediamine, 5-(N-aryl-tropan-3-yl)- and 5-(piperidin-4-yl)-2,4-diamino-pyrimidine, 4'-amino-4-n-propylamino-2-methyl-diphenylsulphone, 5-ethylthioribose, riboflavin-analogues, 1-(3-(2,4-dichlorophenoxy)-1,6-dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamine as the monohydrobromide, 1,6-dihydro-6,6-dimethyl-1-(3-(2,4,5-trichlorophenoxy)-propoxy)-1,3,5-triazine-2,4-diamine as the monohydrochloride, trans-2-(4-(1,1-dimethylethyl)-cyclohexyl)-3-hydroxy-1,4-naphthalenedione, enpiroline, mirincamycin, tripynadine, 3-(4-imidazolyl)- 2-(pivaloylamido)propionylhydrazide, 2-acetylpyridine-thiosemicarbazones and the pyrrolidine derivatives thereof.

However, the use of these substances on their own or in conjunction with one another has the disadvantage that they achieve only a preventive or only temporary effect and the pathogens in question develop resistance more or less quickly; furthermore, many of these compounds have a toxic effect or are effective only in toxic concentrations (PETERS, W, Antimalarial drug resistance: an increasing problem, Br. med. Bull. 38, 187-192, 1982; YOUNG, M.D. and MORE, D.V. Chloroquine resistance in Plasmodium falciparum, Am. J. trop. Med. Hyg. 10, 317-320, 1961; BYGBJERG, I.C. et al., Mefloquin resistance of flaciparum malaria from Tanzania enhanced by treatment, Lancet 1, 21-26 1983; SCHMIDT, L.H.,

Antimalarial properties of floxacrine, a dihydro-acridinedione derivative, Antimicrob. Agents Chemother 16, 475-485, 1979; BRUCE-CHWATT, L.J. Essential malariology, W. Heinemann Medical Books Ltd., London, 1980).

Many of these compounds have undesirable side effects or can be administered only to certain groups of people; furthermore, the very short plasma half-life of some of these substances prevents reasonable prophylactic use (BRUCE-CHWATT et al., Chemotherapy of malaria, 2nd edn. WHO, Geneva 1981; COLBOURNE, M.J., Malaria Prophylaxis for long-term visitors, Comm. Dis. Rep. 35, 3-4, 1983; JIANG, J.B. et al., Antimalarial activity of mefloquine and quinghaosu, Lancet 2, 285-288, 1982.

In view of the increasing resistance to the compounds mentioned above, various combinations of these substances have been used, e.g. pyrimethamine with sulphadoxine (DOBERSTYN, E.B. et al., Single-dose therapy of falciparum malaria using primethamine in combination with diformyldapsone or sulfadoxine, Am. J. trop. Med. Hyg. 25, 14-19, 1976; HALL, A.P. et al., Falciparum malaria cured by quinine followed by sulfadoxine pyrimethamine, Br. med. J. 2, 15-17, 1975; MERKLI, B. et al., The inhibitory effect of a drug combination on the development of mefloquine resistance in Plasmodium berghei, Ann. trop. Med. Parasit. 74, 1-9, 1980).

However, such combinations have not solved the problem of drug resistance and in addition complications have arisen caused by side effects (HURWITZ, E.S. et al., Resistance of Plasmodium falciparum malaria to sulfadoxine-pyrimethamine (Fansidar) in a refugee camp in Thailand, Lancet 1, 1068-70, 1981; PHILLIPS, R.E. et al., Failure of chloroquine-erythromycin and chloroquine-tetracycline combinations in treatment of chloroquinine resistent falciparum malaria in eastern

Thailand, Lancet 1, 300-302, 1984; BJÖRKMAN A. & PHILLIPS - HOWARD, P.A., The epidemiology of drug resistant malaria, Trans. R. Soc. Trop. Med. Hyg. 84, 177-180, 1990).

In addition to the use of antimalarial substances of this kind it has been proposed to treat malaria infection prophylactically by immunisation or vaccination.

However, the use of various preparations from the different plasmodial stages (sporozoites, merozoites, schizonts, gametes) has produced unsatisfactory results, particularly as the result of undesirable autoimmune reactions and non-specific immune responses (TRAGER, W. et al., Immunisation of owl monkey to Plasmodium falciparum with merozoites from cultures of a knobless clone, Parasite Immun. 5, 255, 1983; WERNSDORFER, W.H., Prospect for the development of malaria vaccines, Bull, WHO 59, 335, 1981). Hitherto, an ideal vaccine has not become available (YOUNG, J.F., POSTE G., The prospects for a human malaria vaccine, TIBTECH 6, 63-68, 1988).

In addition to the above-mentioned processes, the use of interferon, e.g. interferon-gamma (IFN-γ), has been proposed both for vaccination (PLAYFAIR, J.H.L., SOUZA, J.B., Recombinant gamma interferon is a patent adjuvant for a malaria vaccine in mice, Clin. Exp. Immunol. 67, 5-10, 1987; HEATH, A.W. et al., Interferon-gamma as an adjuvant in immuno-compromised mice, Immunol. 67, 520-524, 1989), and also for therapeutic treatment (BIENZLE, V. et al., Inhibition of Plasmodium vinckei - malaria in mice by recombinant murine interferon-γ, Acta Tropica 45, 289-290, 1988; MASHESHWARI, R.K. et al., Recombinant human gamma-interferon inhibits simian malaria, Infect. Immun. 53, 628-633, 1986; FERREIRA, A. et al., Inhibition of development of exoerythrocytic forms of malaria parasites by γ-interferon, Science 232, 881-883, 1986; CLARK, I.A. et al., Inhibition of murine malaria

(Plasmodium chabaudi) in vivo by recombinant interferon-γ or tumor necrosis factor, and its enhancement by butylated hydroxyanisole, J. Immunol 139, 3493-3496, 1987; SHEAR, H.L. et al., Role of IFN-γ in lethal and non lethal malaria in susceptible and resistant murine hosts, J. Immunol. 143, 2038-2044, 1989).

In the former case, IFN-γ acted as an adjuvant in conjunction with a suitable antigen; in the second case, although the development of the disease was slowed down in prophylactic use, therapeutic application, i.e. post infectionem, was less effective and was unable to inhibit the multiplication of parasites sufficiently. The combined prophylactic and therapeutic application of IFN-γ had only a cumulative effect. There was no successful treatment in the sense of curative effect in any of the cases since all the animals (mice) died from the malaria infection when treated by any of the three methods, irrespective of how long the treatment lasted and with what dosages the animals were treated (BIENZLE, V. et al., 1988, loc. cit.).

In US Patent 4,915,941 it is proposed to use IFN-γ in conjunction with an antimalarially active substance for preventing malaria infection, in which the administration of this combination does not extend beyond the prepatent phase (liver phase). During this phase it is not possible to diagnose malaria infection.

Surprisingly, it has now been found that a combination consisting of interferon, particularly IFN-γ, and at least one antimalarially effective agent is suitable for treating malaria in the erythrocytic, i.e. clinical phase, and manages to destroy the parasites in the red blood cells (erythrocytes).

The chief difference from the prior art is thus that it is unexpectedly possible to carry out a lasting treatment of clinical malaria, i.e. malaria which can be diagnosed by known methods.

One aspect of the invention provides a method of treating a subject suffering from clinical (erythrocytic) malaria which comprises administering effective amounts of an interferon and at least one antimalarially active substance to the subject.

A further aspect of the invention is use interferon and at least one antimalarially active substance for preparing a pharmaceutical composition for the treatment of erythrocytic, clinical malaria.

Surprisingly, it has also been found that the combined administration of interferon with at least one antimalarially active substance led to a synergistic increase in activity in the treatment of clinical malaria and a lasting cure for the disease, free from recurrence, without any formation of resistance in non-immune individuals.

It was also found, surprisingly, that the administration of the drug combination according to the invention, in addition to the synergistic antimalarial effect, imparts an additional rapid immunity to the particular pathogen, even after a single infection with this pathogen.

Thus, the use of the drug combinations according to the invention to treat clinical malaria achieves a multiple advantage: a lasting curative treatment of the clinically manifest infection without the formation of resistance in non-immune individuals and the rapid formation of immunity to the pathogen in question.

In contrast to the use of the drug combination according to the invention, when administering the particular monosubstances (antimalarially active substance or interferon) hitherto it has only been possible to achieve a slight extension in the survival time compared with the placebo-treated group or, if the combination of IFN-γ plus antimalarial agent was prescribed, it was proposed for use only in the non-clinical prepatent phase.

In some cases, when the antimalarial substance, e.g. chloroquine, was administered in high doses post infectionem, this treatment resulted in survival of a malaria infection; however, no protection was conferred against reinfection with the same strain of pathogen.

Reinfection experiments with the group treated according to the invention, on the other hand, showed that the blood of this group was not infectious and that protection was obtained against reinfection by the same strain of pathogen.

Suitable antimalarial substances for use according to the invention include the known synthetic, semisynthetic and naturally occurring active substances such as those in the above-mentioned groups of compounds, compounds and individual active substances and in the form of combinations thereof. Preferably, the substance is selected from 9-aminoacridines, 4-aminoquinolines, 8-aminoquinolines, biguanides, diaminopyrimidines, quinine salts, sulphonamides, sulfanilamides, antibiotics and/or sulphones. 4-aminoquinolines are a preferred group of antimalarially active substances and chloroquine [(7-chloro-4-(4-diethylamino-1-methylbutylamino)-quinoline] is a favoured compound.

Naturally occurring, synthetic and semisynthetic interferons e.g. of type I and type II and those prepared by genetic engineering using DNA-recombination (e.g. interferons α, β and γ) may be used; interferon γ (IFN-γ) being preferred.

The IFN-γ may be prepared by the known methods of conventional cell cultures of animal or human origin, e.g. according to W.R. BENJAMIN et al., Proc. Natl. Acad. Sci. USA. 79, 5379-5383, 1982; Y.K. YIP et al., Proc. Natl. Acad. Sci. USA 78, 1601-1605, 1981; Y.K. Yip et al., Proc. Natl. Acad. Sci USA 79, 1820-1824, 1982, or by the known technology of DNA-recombination, e.g. according to P.W. GRAY et al., Nature 295, 503-508,

1982; E. RINDERKNECHT et al., J. Biol. Chem. 259, 6790-6797; R. DEVOS et al., Nucl. Acids Res. 10, 2487-2501, 1982; P.W. GRAY, D.V. GOEDDEL, Proc. Natl. Acad. Sci. USA 80 5842-5846, 1983 or according to EP-B-77 670, EP-A-271,824 or according to TANIGUCHI et al., Nature 285, 547-549, 1980; GOEDDEL, D. et al., Nature 287, 411-416, 1980; EP-B-95702, EP-A-280,033.

It is preferable to use IFN-γ, particularly an IFN-γ which can be obtained by DNA-recombination using known methods.

It is known to the average person skilled in the art that natural allelic variations occur specifically in the individual or in different populations and can be manifested by one or more different amino acids or by different nucleotides or DNA sequences. Variations or mutations of this kind, which may also be produced by the known methods of DNA recombination or by controlled mutagenesis, as described for example by P.W. GRAY et al., 1982 loc. cit. and R. DEVOS et al., 1982, loc. cit., comprise single or multiple substitutions, deletions, additions, insertions or inversions. These IFN-γs are therefore included according to the invention.

For immunological reasons it is known to those skilled in the art that it is preferably to use species-specific active substances when using biologically active substances native to the body. For the species-specific use of interferon according to the invention it is therefore preferable to use interferon isolated from the particular species-specific tissues or the nucleic acids (RNA, DNA) isolated from the species-specific tissues or cells to produce the particular interferon by DNA recombination, but in particular it is preferable to use the polypeptide identical to the particular genuine interferon with the known biological spectrum of activity of interferon. Thus, for example, the interferon used for the purposes of the invention in

human beings will preferably be a human IFN, more particularly a human IFN-γ.

The interferon according to the invention may be administered by means of the pharmaceutical or galenic formulations known and used by those skilled in the art for the particular method of administration, but preferably those used for parenteral administration, especially for intravenous, intramuscular, subcutaneous, intracutaneous, intraarticular, intrathecal, intraperitoneal infusion or injection, including continuous infusions or intermittent infusions with the pumps available to those skilled in the art, or the administration by means of micro-encapsulated preparations, e.g. based on liposomes, e.g. according to EP-A-213,523.

For preparing a ready-to-use solution for the administration of interferon according to the invention the expert may use the aqueous infusible and injectable solutions known for this purpose, optionally together with the excipients, carriers and/or stabilising substances known in the art. A ready-to-use solution for the purposes of the invention may for example be prepared by dissolving highly purified interferon in "water for injections" or in phosphate-buffered physiological saline solution (pH 7 to 7.5), optionally supplemented with Tween and/or gelatine or an albumin, before administration, the solution being transferred under sterile conditions into suitable containers (e.g. syringes, ampoules, bags).

The quantity of interferon to be administered for the purposes of the invention will be determined in accordance with the dosages known in the art, the severity of the disease, the response rate and the further course of the disease and side effects. Generally speaking, the dosage must be adjusted according to individual criteria.

The antimalarially active synthetic, semisynthetic

or naturally occurring substances used according to the invention, at least one of which is used with animal or human interferon, may be any of the known antimalarially active agents described, advantageously compounds from the group comprising the 9-aminoacridines, 4-aminoquinolines, 8-aminoquinolines, biguanides, diaminopyrimidines, quinine salts, sulphonamides, sulfanilamides, antibiotics (such as tetracyclines) or sulphones (such as dapsone or 4,4'-diaminodiphenylsulphone), e.g. chloroquine [(7-chloro-4-(4-diethylamino-1-methylbutylamino)-quinoline].

The method of administration and dosage will depend on the therapy plans known for the above-mentioned antimalarial agents, including also liposome-based microencapsulated antimalarial substances, e.g. according to EP-A-213,523 or EP-A-152,379 and also, for example, according to EP-A-354,442 or EP-B-56,781, to name just some of the numerous published patent literature.

Another aspect of the invention provides a pharmaceutical composition for the treatment of clinical (erythrocytic) malaria comprising interferon and at least one antimalarially active substance. Preferred interferons and antimalarially active substances are those mentioned above.

The pharmaceutical composition according to the invention comprising least one substance with an antimalarial activity and an interferon can be used for simultaneous administration of the two different types of active substance (antimalarial substance, interferon) or for consecutive or sequential administration by suitable route, the individual active substances being provided and administered either separately, e.g. in the form of a "kit-of-parts" or directly together, in terms of space and time. The active substance components which are present separately or either indirectly or directly together may be provided both as dry substances

and as solutions, whilst microencapsulated forms are also possible in which the active substance components may be used directly together, indirectly as a liposome mixture or as separate systems for administration. It is advantageous for the two active substance components, the antimalarial drug and interferon, to be administered simultaneously, e.g. at the same site in the same excipient.

Legend relating to Figure 1:
Course of a Plasmodium vinckei malaria in BALB/c mice after different treatments (n=6 for each treated group) given, once a day, 80 µg of chloroquine (o_____o), 1 x 10^4U IFNγ (o----o), a combination of both active substances (o....o) or as the control group phosphate buffered saline (PBS) plus murine albumin (o-.-.-.o) 3 days before till 7 days after the infection. The average values of the parasitaemias are shown.

Example

A. Infection:

In all the experiments, female BALB/c mice 8 to 10 weeks old (Federal Health Institute Berlin) were used. The mice were infected with P. vinckei by intraperitoneal injection of 10^5 parasitised erythrocytes suspended in 100 µl of phosphate buffered saline (PBS). For the reinfection experiments, P. vinckei and P. berghei were administered in the same way. Three days post infectionem, thin blood slides were prepared and stained with giemsa dye in order to determine the parasitaemia. The statistical analysis was carried out using the Wilcoxon test.

B. Treatment with chloroquine:

Groups of six mice infected with P. vinckei were treated on the day of infection with varying doses of

chloroquine (Bayer, Leverkusen) in the form of a single intraperitoneal administration in the dosage range from 40 µg to 300 µg per mouse, diluted in 100 µl PBS. The control animals were given only PBS. The untreated animals (control animals) succumbed to the infection after an average time of 8 days. In the mice treated with a high dosage of 300 µg of chloroquine, only a low and delayed parasitaemia was observed and all the mice survived. By comparison, of the group given 120 µg of chloroquine, only two mice survived. All the mice treated with 40 or 80 µg of chloroquine died within 11 days post infectionem. A significant ($P<0.05$) increase in the survival time compared with the controls was observed in the mice treated with 80 µg of chloroquine, but not in those given 40 µg of chloroquine. Therefore, 80 µg (4 mg/kg) was chosen as the subcurative chloroquine treatment for the remaining investigations.

C. Treatment with IFN-γ:

In this group (n=6) the mice were treated with recombinant murine IFN-γ from E. coli (Genentech Inc. South San Francisco, California, or produced according to P.W. GRAY, D.V. GOEDDEL, Proc. Natl. Acad. Sci. USA 80, 5842-5846, 1983) with a specific activity of 1.9×10^7 U/mg of protein dissolved in PBS containing 0.1% murine albumin. The daily intraperitoneal administrations of 100 µl with either 10^4 U IFN-γ per mouse or 5×10^4U IFN-γ per mouse were given over a period of 11 successive days, the treatment beginning 3 days before infection and ending 7 days after infection. The control animals were given PBS plus murine albumin (0.1%). The administration of IFN-γ significantly ($P<0.05$) delayed the outbreak of any clear parasitaemia and increased the survival time. In those mice which were given 1×10^4 U IFN-γ per day over a period of 11 days, the survival time was increased by only 1 day

compared with the controls and all the mice died.

However, of those mice which were given 5×10^4 U IFN-γ per day, three of the six mice survived the P. vinckei malaria.

D. Treatment with IFN-γ plus chloroquine:

This third group of mice were treated with 1×10^4 U IFN-γ per day (as described in C.) in conjunction with 80 µg of chloroquine (as described in B.) and compared both with the mice which were given these two substances separately and with the control group (Fig. 1).

The control mice died after 9.0 days (average), the chloroquine-treated mice died after 10.25 days (average) and the IFN-γ treated mice died 10.0 days (average) post infectionem. In the group given the combined treatment, the parasitaemia only became obvious on day 5 or day 6 after the infection, as against day 3 or day 4 in the other groups. In the mice treated with the combination, the peak parasitaemia was achieved on day 12.

One out of six mice given the combined treatment died on day 11. In three of the five surviving mice there was a second smaller peak of parasitaemia which appeared approximately 18 days after the infection. On day 22 all five mice had negative findings and remained free from parasites for at least five weeks (until the reinfection experiments).

E. Reinfection experiments:

Those mice which survived the P. vinckei malaria thanks to the combined treatment with IFN-γ and chloroquine as described under D., were reinfected with the same strain of P. vinckei five to ten weeks after the first infection.

None of the mice developed any recognisable parasitaemia within an evaluation period of more than ten weeks. However, crisis forms were visible under the microscope. The blood from these mice was then injected

into original, uninfected mice, whereupon no parasitaemia developed. By contrast, those mice which had been cured with high doses of chloroquine (as described under B.) showed no resistance to reinfection with the same strain.

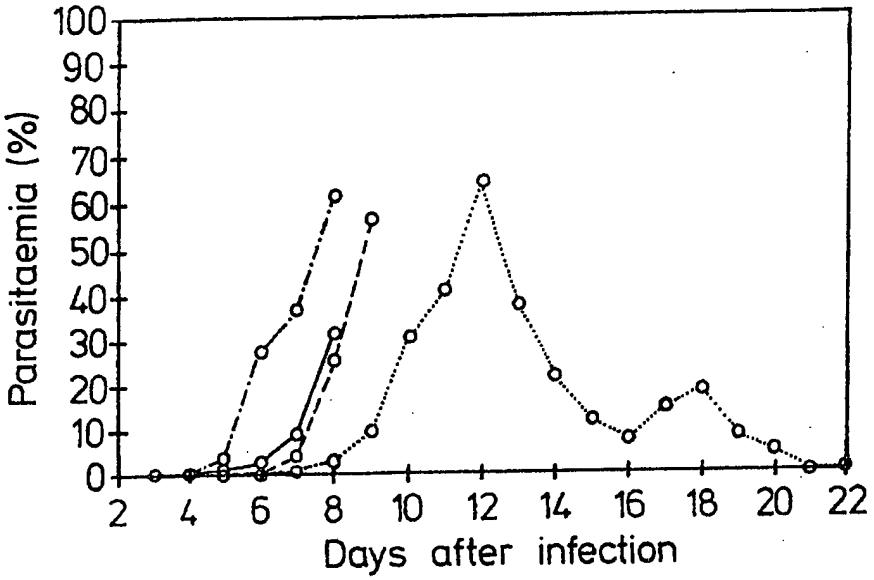
In order to determine whether this immunity achieved with the combined treatment is specific to the strain, the surviving mice treated with the combination were infected either with P. vinckei, P. berghei or with a combination of P. vinckei plus P. berghei in this sequence and compared with control mice infected with the same parasites.

With regard to the course of the parasitaemia, the immune mice infected with P. berghei or P. vinckei plus P. berghei showed a similar tendency compared with a control group of original BALB/c mice infected with P. berghei. The groups of original mice which had been infected either with P. vinckei or with both strains showed an earlier increase in parasitaemia, caused by the unhindered multiplication of P. vinckei (Table 1).

Table 1: Reinfection experiments. Course of the average parasitaemias (range) in three groups of original mice which were infected with either Plasmodium berghei anka or Plasmodium vinckei or a combination of P. berghei plus P. vinckei (n=6 in each case) and in three groups of immune mice which were infected with either P. berghei or P. vinckei or a combination of P. berghei plus P. vinckei (n=6 in each case).

Days after infection	4	5	6
original mice infected with P. berghei	1 % (0.1-3)	3.5 % (1-5)	5.5 % (4-7)
immune mice infected with P. berghei	0.5 % (0.1-1)	3 % (2-6)	6.5 % (5-11)
original mice infected with P. vinckei	1 % (0.5-2)	5 % (3-10)	16 % (8-23)
immune mice infected with P. vinckei	0 % (0-0)	0 % (0-0)	0 % (0-0)
original mice infected with P. berghei and P. vinckei	3 % (3-4)	9 % (7-10)	12.5 % (11-14)
immune mice infected with P. berghei and P. vinckei	0.5 % (0.1-2)	4.5 % (2-7)	8.5 % (5-11)

FIG. 1



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